

**We Claim**

- 1) A process for the enhanced recovery of recombinant insulin comprising, treating the expression broth/culture medium containing the expressing cells either with water miscible organic solvents and/or with salts and/or with amino acids and/or at different pH conditions, wherein the concentration of the insulin *in solution* is higher, than had the expression broth or culture medium not been treated with the said conditions.
- 2) A process for the enhanced recovery of recombinant insulin comprising, treating the expression broth/culture medium containing the expressing cells either with urea, wherein the concentration of the insulin *in solution* is higher, than had the expression broth or culture medium not been treated with urea.
- 3) A process according to claim 1 where the treatment is carried out from about pH 2.0 to about pH 5.0
- 4) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with Arginine, at a pH from about 2 to about 4.
- 5) A process according to claim 4 wherein the concentration of arginine is from about 1 to about 100 mM.
- 6) A process according to claim 4 wherein the arginine concentration is from about 50 mM to about 100 mM.
- 7) A process according to claim 4 wherein the arginine concentration is from about 70 mM to about 100 mM.
- 8) A process according to claim 4 wherein the arginine concentration is about 100 mM.
- 9) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with Aspartic acid, at a pH from about 2 to about 4.
- 10) A process according to claim 9 wherein the concentration of aspartic acid is from about 1 mM to about 100 mM.
- 11) A process according to claim 9 wherein the aspartic acid concentration is from about 50 mM to about 100 mM.
- 12) A process according to claim 9 wherein the aspartic acid concentration is from about 70 mM to about 100 mM.
- 13) A process according to claim 9 wherein the aspartic acid concentration is about 100 mM.
- 14) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with calcium chloride, at a pH from about 2 to about 4.
- 15) A process according to claim 14 wherein the concentration of calcium chloride is from about 1 mM to about 100 mM.
- 16) A process according to claim 14 wherein the calcium chloride concentration is from about 50 mM to about 100 mM.
- 17) A process according to claim 14 wherein the calcium chloride concentration is from about 70 mM to about 100 mM.
- 18) A process according to claim 14 wherein the calcium chloride concentration is about 100 mM.
- 19) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with isopropanol, at a pH from about 2 to about 5.

- 20) A process according to claim 19 where the concentration of isopropanol is from about 1 percent to about 50 percent.
- 21) A process according to claim 19 where the isopropanol concentration is from about 10 percent to about 50 percent.
- 5 22) A process according to claim 19 where the isopropanol concentration is from about 30 percent to about 50 percent.
- 23) A process according to claim 19 where the isopropanol concentration is from about 40 percent to about 50 percent.
- 24) A process according to claim 19 where the isopropanol concentration is about 20 percent.
- 10 25) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with methanol, at a pH from about 2 to about 5.
- 26) A process according to claim 25 where the concentration of methanol is from about 1 percent to about 50 percent.
- 15 27) A process according to claim 25 where the methanol concentration is from about 10 percent to about 50 percent.
- 28) A process according to claim 25 where the methanol concentration is from about 30 percent to about 50 percent.
- 29) A process according to claim 25 where the methanol concentration is from about 40 percent to about 50 percent.
- 20 30) A process according to claim 25 where the methanol concentration is about 20 percent.
- 31) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with ethanol, at a pH from about 2 to about 5.
- 25 32) A process according to claim 31 where the concentration of ethanol is from about 1 percent to about 50 percent.
- 33) A process according to claim 31 where the ethanol concentration is from about 10 percent to about 50 percent.
- 34) A process according to claim 31 where the ethanol concentration is from about 30 percent to about 50 percent.
- 30 35) A process according to claim 31 where the ethanol concentration is from about 40 percent to about 50 percent.
- 36) A process according to claim 31 where the ethanol concentration is about 20 percent.
- 37) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with tertiary-butanol, at a pH from about 2 to about 5.
- 35 38) A process according to claim 37 where the concentration of tertiary-butanol is from about 1 percent to about 50 percent.
- 39) A process according to claim 37 where the tertiary-butanol concentration is from about 10 percent to about 50 percent.
- 40 40) A process according to claim 37 where the tertiary-butanol concentration is from about 30 percent to about 50 percent.
- 41) A process according to claim 37 where the tertiary-butanol concentration is from about 40 percent to about 50 percent.
- 45 42) A process according to claim 37 where the tertiary-butanol concentration is about 20 percent.

- 43) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with acetonitrile, at a pH from about 2 to about 5.
- 44) A process according to claim 43 where the concentration of acetonitrile is from about 1 percent to about 50 percent.
- 5 45) A process according to claim 43 where the acetonitrile concentration is from about 10 percent to about 50 percent.
- 46) A process according to claim 43 where the acetonitrile concentration is from about 30 percent to about 50 percent.
- 47) A process according to claim 43 where the acetonitrile concentration is from about 40 percent to about 50 percent.
- 10 48) A process according to claim 43 where the acetonitrile concentration is about 20 percent.
- 49) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with dimethylformamide.
- 15 50) A process according to claim 49 where the concentration of dimethylformamide is from about 1 percent to about 50 percent.
- 51) A process according to claim 49 where the dimethylformamide concentration is from about 10 percent to about 50 percent.
- 52) A process according to claim 49 where the dimethylformamide concentration is from about 30 percent to about 50 percent.
- 20 53) A process according to claim 49 where the dimethylformamide concentration is from about 40 percent to about 50 percent.
- 54) A process according to claim 49 where the dimethylformamide concentration is about 20 percent.
- 25 55) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with dimethylsulfoxide, at a pH from about 2 to about 5.
- 56) A process according to claim 55 where the concentration of dimethylsulfoxide is from about 1 percent to about 50 percent.
- 30 57) A process according to claim 55 where the dimethylsulfoxide concentration is from about 10 percent to about 50 percent.
- 58) A process according to claim 55 where the dimethylsulfoxide concentration is from about 30 percent to about 50 percent.
- 59) A process according to claim 55 where the dimethylsulfoxide concentration is from about 40 percent to about 50 percent.
- 35 60) A process according to claim 55 where the dimethylsulfoxide concentration is about 20 percent.
- 61) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with ethylene glycol, at a pH from about 2 to about 5.
- 40 62) A process according to claim 61 where the concentration of ethylene glycol is from about 1 percent to about 50 percent.
- 63) A process according to claim 61 where the ethylene glycol concentration is from about 10 percent to about 50 percent.
- 45 64) A process according to claim 61 where the ethylene glycol concentration is from about 30 percent to about 50 percent.

- 65) A process according to claim 61 where the ethylene glycol concentration is from about 40 percent to about 50 percent.
- 66) A process according to claim 61 where the ethylene glycol concentration is about 20 percent.
- 5 67) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with propylene glycol, at a pH from about 2 to about 5.
- 68) A process according to claim 67 where the concentration of ethylene glycol is from about 1 percent to about 50 percent.
- 10 69) A process according to claim 67 where the propylene glycol concentration is from about 10 percent to about 50 percent.
- 70) A process according to claim 67 where the propylene glycol concentration is from about 30 percent to about 50 percent.
- 71) A process according to claim 67 where the propylene glycol concentration is from about 40 percent to about 50 percent.
- 15 72) A process according to claim 67 where the propylene glycol concentration is about 20 percent.
- 73) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with acetic acid, at a pH from about 2 to about 5.
- 20 74) A process according to claim 73 where the concentration of acetic acid is from about 1 percent to about 50 percent.
- 75) A process according to claim 73 where the acetic acid concentration is from about 10 percent to about 50 percent.
- 76) A process according to claim 73 where the acetic acid concentration is from about 30 percent to about 50 percent.
- 25 77) A process according to claim 73 where the acetic acid concentration is from about 40 percent to about 50 percent.
- 78) A process according to claim 73 where the acetic acid concentration is about 20 percent.
- 30 79) A process as in claim 1 comprising, treatment of the expression broth/culture medium containing the expressing cells with dioxan, at a pH of about 2 to about 5.
- 80) A process according to claim 79 where the concentration of dioxan is from about 1 percent to about 20 percent.
- 81) A process according to claim 79 where the dioxan concentration is from about 10 percent to about 20 percent.
- 35 82) A process according to claim 79 where the dioxan concentration is from about 15 percent to about 20 percent.
- 83) A process according to claim 79 where the dioxan concentration is about 20 percent.
- 84) A process as in claim 2 comprising, treatment of the expression broth/culture medium containing the expressing cells with urea, at about pH of about 2 to about 5.
- 40 85) A process according to claim 84 where the concentration of urea is from about 1M to about 6M.
- 86) A process according to claim 84 where the urea concentration is from about 3M to about 6M.
- 45 87) A process according to claim 84 where the urea concentration is 6M.

- 88) A process for the enhanced recovery of recombinantly expressed insulin into solution, comprising, treatment of the expression broth/culture medium containing the expressing cells with isopropanol at pH about 2, followed by removal of the supernatant, followed by extraction(s).
- 5 89) A process for the enhanced recovery of recombinantly expressed insulin into solution according to claim 88, comprising at least two extraction steps with isopropanol, of the expression broth/culture medium containing the expressing cells, at pH about 2.
- 90) A process according to claim 89 wherein the concentration of isopropanol used in each step is same or different.
- 10 91) A process according to claim 90 wherein the concentration of isopropanol used in each step is different.
- 92) A process according to claim 90 wherein the concentration of isopropanol used in each step is same.
- 93) A process according to claim 92 wherein the concentration of isopropanol used is, from about 1 percent to about 50 percent.
- 15 94) A process according to claim 92 wherein the isopropanol concentration is from about 10 percent to about 50 percent.
- 95) A process according to claim 92 wherein the isopropanol concentration is from about 30 percent to about 50 percent.
- 20 96) A process according to claim 92 wherein the isopropanol concentration is from about 40 percent to about 50 percent.
- 97) A process according to claim 92 wherein the isopropanol concentration is about 20 percent.
- 25 98) A process for the enhanced recovery of recombinantly expressed insulin into solution, comprising of sequential extraction of the expression broth/culture medium containing the expressing cells with at least two of following steps, but not necessarily in the same order :
- a) citrate buffer at about pH 2 to about pH 5
- b) about 0.2 M to about 1.0 M sodium chloride at about pH 2 to about pH 5
- 30 c) about 10 percent to about 50 percent isopropanol at about pH 2 to about pH 5
- d) about 1M to about 5M urea at about pH 2
- e) about 1M to about 5M urea at about pH 4.
- 99) A process for the isolation and purification of insulin from recombinant sources consisting of subjecting the expression broth/culture medium containing the expressing cells to chromatography in an expanded mode.
- 35 100) A process as in claim 99 wherein the chromatography is ion exchange chromatography.
- 101) A process according to claim 100 wherein the ion exchange chromatography is cation exchange chromatography.
- 40 102) A process according to claim 101 in which the fermentation broth/culture medium containing the expressing cells is diluted with buffer at about pH 3, then fed to column packed with Streamline-SP cation exchange resin, followed by washing with buffer at about pH 4 and then followed by elution with buffer at about pH 7.5.
- 103) A process according to claim 101, wherein the dilution buffer is citrate buffer, wash buffer is citrate buffer.
- 45 104) A process according to claim 101, wherein the elution buffer is Tris HCl buffer

- 105) A process according to claim 101, wherein the dilution buffer is citrate buffer, wash buffer is citrate buffer and the elution buffer is Tris HCl buffer
- 106) A process for the isolation and purification of insulin from recombinant sources consisting of treatment of the expression broth/culture medium containing the  
5 expressing cells with water miscible organic solvent followed by chromatography in an expanded bed mode.
- 107) A process as in claim 106 wherein the chromatography is ion exchange chromatography.
- 108) A process according to claim 107, wherein the ion exchange chromatography is  
10 cation exchange chromatography.
- 109) A process according to claims 108 wherein the said water miscible organic solvent is isopropanol.
- 110) A process according to claim 109 wherein the treatment is carried out with isopropanol at a concentration of about 5 percent to about 90 percent and at a pH of  
15 about 1 to about 4.
- 111) A process according to claim 109 wherein the treatment is carried out with isopropanol at a concentration of about 10 percent to about 50 percent and at a pH of about 3.
- 112) A process according to claim 109 wherein the treatment is carried out with isopropanol at a concentration of about 20 percent to about 50 percent and at a pH of  
20 about 3.
- 113) A process according to claim 109 wherein the treatment is carried out with isopropanol at a concentration of about 20 percent and at a pH of about 3.
- 114) A process according to claim 108 wherein the treatment is carried out with isopropanol at a concentration of about 20 percent and pH of about 3.  
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- 115) A process according to claims 108 wherein the said water miscible organic solvent is methanol.
- 116) A process according to claim 115 wherein the treatment is carried out with methanol at a concentration of about 5 percent to about 90 percent and at a pH of  
30 about 1 to about 4.
- 117) A process according to claim 115 wherein the treatment is carried out with methanol at a concentration of about 10 percent to about 50 percent and at a pH of about 3.
- 118) A process according to claim 115 wherein the treatment is carried out with methanol at a concentration of about 20 percent to about 50 percent and at a pH of  
35 about 3.
- 119) A process according to claim 115 wherein the treatment is carried out with methanol at a concentration of about 20 percent and at a pH of about 3.
- 120) A process according to claim 115 wherein the treatment is carried out with methanol at a concentration of about 20 percent and pH of about 3.  
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- 121) A process according to claims 108 wherein the said water miscible organic solvent is dimethylformamide.
- 122) A process according to claim 121 wherein the treatment is carried out with dimethylformamide at a concentration of about 5 percent to about 70 percent and at a  
45 pH of about 1 to about 4.

- 123) A process according to claim 121 wherein the treatment is carried out with dimethylformamide at a concentration of about 10 percent to about 50 percent and at a pH of about 3.
- 5 124) A process according to claim 121 wherein the treatment is carried out with dimethylformamide at a concentration of about 20 percent to about 50 percent and at a pH of about 3.
- 125) A process according to claim 121 wherein the treatment is carried out with dimethylformamide at a concentration of about 20 percent and at a pH of about 3.
- 10 126) A process according to claim 121 wherein the treatment is carried out with dimethylformamide at a concentration of about 20 percent and pH of about 3.
- 127) A process, according to any of the above claims in which the recombinant source expressing the insulin is yeast.
- 128) A process according to claim 127 in which the yeast include genera Hansenula, Saccharomyces, Pichia, Kluyveromyces.
- 15 129) A process according to claim 127, in which the insulin expressed by a yeast strain is secreted into the extracellular medium.